

# **Report as of FY2008 for 2008MT172B: "Student Fellowship: How susceptible to chlorine disinfection are detached biofilm particles?"**

## **Publications**

Project 2008MT172B has resulted in no reported publications as of FY2008.

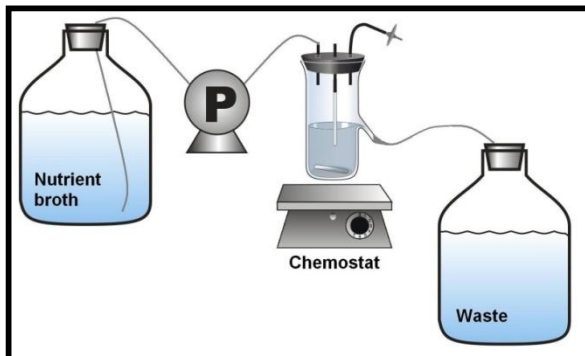
## **Report Follows**

## An update:

### How susceptible to chlorine disinfection are detached biofilm particles?

#### Background:

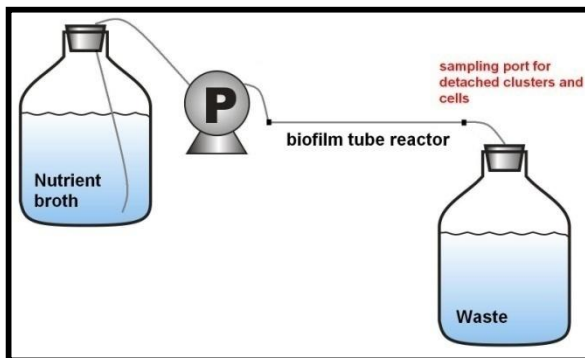
Previously, disinfection experiments have been done with *Salmonella typhimurium* cultures (update May 2008). In June we started performing experiments with *Burkholderia cepacia* under identical conditions.



*B. cepacia* FS-3 is able to grow in the defined medium consisting of phosphate buffer, 0.1 g/L glucose, 0.018g/L  $\text{NH}_4\text{Cl}$ , and  $\text{MgSO}_4$ . Initial experiments have found that the maximum growth rate in this medium is  $0.148 \pm 0.024 \text{ h}^{-1}$  which results in a doubling time of about 6 hours.

Samples are taken by removing the lid from the chemostat and using a sterile pipette. The diluted chemostat culture contained single cells as well as clusters up to 100 cells. Clusters

bigger than 100 cells were rare. Most cells are in small clusters of 2 to 5 cells.



*B. cepacia* FS-3 has been found to establish a biofilm in the silicone tubing of the biofilm tube reactor. The reactor is inoculated with a sterile syringe filled with 10 ml fresh overnight culture followed by a 3 hour attachment period without flow. After this period, the flow is set to 0.9 ml/min resulting in a residence time of about 4 minutes.

Samples of detached cells are removed by collecting effluent in a sterile tube, while samples of biofilm are removed by cutting the

silicone tubing into small (4 cm long) pieces.

Cluster and cells distribution in effluent samples is very similar to what we observed in the chemostat samples. The mechanically detached and homogenized biofilm was also subject to image analysis. Again, a similar pattern to chemostat culture and tube reactor effluent exists.

#### General Information about experiments:

- 30 min. exposure to Chlorine
- Chlorine added to samples according to a standard curve made with increasing amounts of fresh Chlorine stock in medium without N and C source
- Neutralization of Sodium hypochlorite with Sodium thiosulfate
- Detection limit was 5 CFU/ml or lower for all experiments
- Disaggregation: shear Homogenization at 20,000 rpm for 1 min.

## Discussion

Cells and clusters of the chemostat culture are very susceptible to low amounts of Chlorine. In the chemostat, growth is occurring exponentially which is known to make cells more susceptible to disinfection than cells in the stationary phase.

The cluster size distribution of detached biofilm particles and chemostat particles are almost identical and initial cells numbers are very similar. Despite this, detached biofilm clusters are less susceptible to Chlorine. This may be due to an increased amount of extracellular polymeric substances surrounding clusters and cells. This hypothesis is supported by the fact that tube reactor effluent samples consume more free chlorine during the experiment than chemostat samples (for example: 0.86 ppm more Chlorine consumed for tube reactor effluent (on average) upon addition of 2 ppm). This indicates that more (organic) substances in the sample react with the available Chlorine making less of it available for disinfection.

As anticipated, the biofilm is less susceptible to disinfection. The attachment to a surface (silicone tubing) may be protective to the biofilm since Chlorine can attack the biofilm from only one side.

Intuitively, biofilm samples are a more resistant to disinfection than detached biofilm clusters. Initial cell numbers are slightly higher than initial cell numbers for the chemostat culture or the tube reactor effluent.

